Metabolism of human apolipoproteins A-I and A-II: compartmental models

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Abstract The metabolism of radioiodinated apolipoproteins (apo) A-I and A-II have been examined using the techniques of compartmental modeling. The model for apoA-I contains two plasma compartments decaying at different rates. One component of apoA-I has a residence time of 3.8 days and the second has a residence time of 6.1 days. In contrast, the apoA-II model has only one plasma component, with a residence time of 5.5 days, which decays through two distinct pathways. Twenty-seven percent of apoA-II decays through a pathway that takes 1.1 days longer to reach the urine than the remaining 73% which decays through the more direct path. These differences in the metabolism exist in both male and female populations. Comparison of fasting and nonfasting concentrations of apoA-I revealed that apoA-I concentration was elevated 0.5 standard deviations in the nonfasting samples while there was no significant difference in the apoA-II concentrations. The fasting apoA-I concentrations were found to be less stable over the study period when compared to fasting apoA-II concentrations. These findings are interpreted as indicating that apoA-I and apoA-II each have a separate metabolism which overlaps when they are present on the same lipoprotein particle. Furthermore, these findings are consistent with the concept that apoA-I metabolism is influenced more by perturbations such as dietary modulation.—**Zech, L. A., E. J. Schaefer, T. J. Bronzert, R. L. Aamodt, and H. B. Brewer, Jr.** Metabolism of human apolipoproteins A-I and A-II: compartmental models. *J. Lipid Res.* 1983. **24:** 60–71.

Supplementary key words kinetic model

Recent recognition of the inverse correlation between high density lipoprotein (HDL) cholesterol levels and the development of premature cardiovascular disease (1–8) has kindled considerable interest in this group of lipoproteins. It has been proposed that HDL is involved in the removal and transport of cholesterol from peripheral cells to the liver, the principle organ responsible for the removal of cholesterol from the body. This process has been termed "reverse cholesterol transport" (9). Several mechanisms have been proposed for the primary role of HDL in protecting against cardiovascular disease including efficient reverse cholesterol transport and competitive binding with low density lipoprotein (LDL) at the LDL high affinity receptor site (10–12). Low levels of HDL-cholesterol may also be associated with defects in the metabolism of triglyceride-rich particles. Abnormal metabolism of these lipoprotein particles may lead to decreased input of lipid and apolipoprotein constituents into HDL, and the ultimate accumulation of atherogenic particles (13). Recent interest in triglyceride metabolism and the recognition of the inverse correlation between plasma triglyceride concentrations and HDL-cholesterol levels (2, 3, 14, 15) has further stimulated interest in HDL metabolism. The role of HDL as an acceptor of metabolic products following the metabolism of triglyceride-rich lipoproteins in man (16) and rat (17), and its role as a reservoir of cofactors for the enzymic hydrolysis of triglyceride-rich lipoproteins have been extensively investigated (18, 19).

In addition, several studies have focused on the physiochemical characterization (20–22) and metabolism (12, 23–25) of HDL. High density lipoproteins have been shown to be a polydisperse collection of cholesterol-rich lipoprotein particles varying in hydrated density and containing several apoproteins including apoA-I, apoA-II, apoC-I, apoC-II, apoC-III, apoD, and apoE (26–30). Apolipoproteins A-I and A-II constitute approximately 90% of the protein moiety of HDL.

The sites of synthesis of the lipid component of HDL have not been definitively identified. In the rat, bilayered disk-shaped lipoproteins with the hydrated density of HDL have been detected in mesenteric lymph (31) and in liver perfusate (32). It has been suggested that these nascent HDL particles "mature" following the esterification of cholesterol through the action of lecithin:cholesterol acyltransferase (LCAT), and the accu-

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins.

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mulation of cholesterol ester into the hydrophobic core of the particles (32). A second major transfer of lipid and apolipoproteins to HDL follows the hydrolysis of triglyceride-rich lipoproteins of liver and intestinal origin by lipoprotein lipase (16, 19, 33–35).

The sites of synthesis of HDL apoproteins have been extensively investigated in the rat. ApoA-I has been shown to be synthesized in the intestine in studies employing intestinal perfusion with radiolabeled amino acids (36, 37) and by direct investigation of apoA-I in intestinal cells with immunofluorescent techniques (38). Liver perfusion studies in the rat have also shown this organ to be a site of apoA-I synthesis (32). Windmueller, Herbert, and Levy (36) have quantified the fraction of apoA-I synthesized in rat liver to be approximately 40–60% of total apoA-I production. The presence of both apoA-I and apoA-II has been demonstrated in human intestinal cell by the immunoperoxidase methods (39).

The metabolism of HDL has been investigated by analysis of the decay of HDL radiolabeled in its apolipoprotein constituents. Studies were performed by Blum et al. (23) using a labeled subfraction of HDL (d 1.09–1.21 g/ml). These investigators constructed a compartmental model and used it to calculate production rate and fractional catabolic rate for HDL. In these studies the terminal slopes of the specific activity decay of apoA-I and apoA-II were parallel. Blum et al. (23) concluded from these studies that apoA-I and apoA-II had equivalent fractional catabolism. Shepherd et al. (40) examined the kinetics of radiolabeled apoA-I and apoA-II in several studies and concluded that there was a difference in catabolism of radiolabeled apoA-I and apoA-II within HDL. This difference in catabolism was attributed to methodology with respect to recombination of labeled apoA-I in vitro with HDL. This finding resulted in the calculation of two production (synthesis) rates for apoA-I using both the apoA-I residence time and apoA-II residence time (41). Fidge et al. (42) also investigated the kinetics of apoA-I and apoA-II by examining the specific activity of these apolipoproteins in HDL following the injection of radioiodinated HDL. Because of the small number of normal subjects investigated, they concluded that the differences in apoA-I and apoA-II specific activity decay that did not reach statistical significance were important. Kushwaha, Foster, and Hazzard (43) have recently reported differences in the metabolism of HDL apoA-I and apoA-II following the injection of radiolabeled HDL into pigtail monkeys.

In this study we report the analysis of apolipoprotein kinetics following the direct injection of radiolabeled apolipoproteins into normal humans. Preliminary analysis of these data utilizing the area under the radioactivity decay curve indicated a difference between the metabolism of apoA-I and apoA-II (44) that was inconsistent with the present HDL model (23). Therefore, models for the metabolism of apolipoproteins A-I and A-II were developed to serve as an aid in our further understanding of HDL metabolism. Metabolism of HDL in males and females was also compared and contrasted by examining their apoA-I and apoA-II metabolism.

METHODS

Twenty normal control subjects were studied on a metabolic ward. All subjects were instructed not to change their level of activity during the study and were placed on an iso-weight diet consisting of 20% protein, 40% carbohydrate, 40% fat, polyunsaturated:saturated fat ratio = 0.1–0.3, and containing 300 mg cholesterol/day. Over the period of study weight fluctuated by less than 1 kg in each subject. Two days prior to the injection of radiolabeled apolipoproteins, each subject began receiving 1 g/day of supersaturated potassium iodide and 900 mg/day of ferrous sulfate in divided doses. These medications were continued through each study. Informed consent was obtained from all subjects. Following the injection of radioiodinated apolipoproteins, plasma samples were collected in 0.1% EDTA. Plasma and urine radioactivity was determined using a Packard 3375 Gamma Spectrometer (Downers Grove, IL). Whole-body radioactivity was determined using the NIH Whole-Body Counter (45).

Preparation and quantification of apolipoproteins

ApoA-I and apoA-II were isolated from normal HDL (1.090–1.21 g/ml) by column chromatography as previously described (44). Both apolipoproteins were radioiodinated with ^{131}I or ^{125}I by the method of McFarlane (46) utilizing the conditions of Schaefer et al. (44). The physiochemical properties were examined and the radiolabeled proteins were prepared for injection as previously described (44). Lipid and lipoprotein quantification was performed using the Lipid Research Clinics Methodology (47) and apolipoprotein concentrations were quantified by radial immunodiffusion under the conditions and using the standards previously reported (44).

Data normalization

Plasma radioactivity was normalized by dividing the value at each time point by the initial value determined for the 10-min sample. The urine radioactivity was normalized by dividing the value at each time point by the total injected dose. Whole-body radioactivity was nor-

malized by dividing the value at each time point by the initial time point.

A goal of this study was to determine a best[3] estimate of the parameters describing apolipoprotein kinetics in the population of normal humans. Therefore, this group of individuals was not considered as individuals but as a single sample from the population of interest. Each subject was given equal weight in this estimate. The mean population plasma, urine, and whole-body radioactivity was estimated by using the arithmetic mean[4] of the appropriate normalized data at each time point over all subjects. Thus, at time t, the appropriate radioactivity was:

$$F(t) = \frac{\sum_{i=1}^{n} f_i(t)}{n} \simeq \left(\prod_{i=1}^{n} f_i(t)\right)^{1/n} \qquad \text{Eq. } 1)$$

The variance was estimated by determining the standard error for the arithmetic mean at each time point.

$$V(t) = \frac{\sum_{i=1}^{n} [F(t) - f_i(t)]^2}{n(n-1)} \qquad \text{Eq. } 2)$$

The grand mean, equation *3*, was used as an estimate of the normal population plasma concentration [ ] where N_k is the number of determinations for subject k and n is the number of subjects studied.

$$\text{G.M.} = \sum_{k=1}^{n} \sum_{i=1}^{N_k} [\ ]_{ik} / \sum_{k=1}^{n} N_k \qquad \text{Eq. } 3)$$

The plasma concentration [ ] data was also examined in each subject for changes related to the duration of the study using a linear regression model, equation *4*.

$$[\ ] = B_0 + B_1 t \qquad \text{Eq. } 4)$$

This statistical model, when applied to n subjects, results in three parameter sets, intercepts B_{i0}, slopes B_{i1}, and residuals (errors) e_i where,

$$e_i = \text{calculated values}_i - \text{observed values}_i.$$

In addition, variances for all three statistics B_0, B_1, and e_i, were estimated using the SAAM simulator (48) and the following equation where n = number of residuals and k = 2.

$$S = \frac{1}{n - (k+1)} \sum_{i=1}^{n} e_1^2 \qquad \text{Eq. } 5)$$

Standard residuals were calculated by dividing each residual by the square root of the appropriate variance S. The distribution of residuals was plotted against the independent parameter time (t) for each subject. These plots were examined to detect transcription errors, clusters, heteroscedasticity, and the presence of systematic trends. Serial correlation of residuals was examined using a Durbin-Watson d statistic (49). The assumption that the standard residuals are normally distributed with mean zero and variance one was examined using the Kolmogorov-Smirnov test (50, 51). Any distribution with more than a 5% chance of being normal was accepted as normal. Sensitivities were calculated using an extension of the methods as previously cited (52).

Model development

The general techniques of compartmental analysis were applied to the data resulting in development of compartmental models for both apoproteins A-I and A-II kinetics in normal humans. In these models, a compartment represents a distinct, homogeneous, well-mixed pool of material. Each compartment in the model can have material flowing in or out, and this flow of material is designated by an arrow. Arrows flowing into a compartment that do not originate at another compartment represent flow into the model. Arrows that do not terminate at any compartment represent material that is leaving the model. Associated with each arrow are two numbers representing the rate of flow of material [R(I, J)] and the fractional rate of flow of material in that portion of the model [L(I, J)]. Associated with each compartment in the model are two numbers representing the amount of material in that compartment [F(I)] and the amount of time each unit of material resides in a compartment before an irreversible loss occurs [T(K)]. Each model is determined by the number of compartments, the connectivity of the compartments, and the number of compartments representing a physiological space. When using models to compare groups of data, these three model characteristics are compared and related to the physiologic system under examination (53). Simulation of each compartmental model was carried out using the SAAM simulator (48) on a VAX-11/780 computer system (Digital Equipment Corp.). Utilizing these compartmental models, residence time (the average amount of time an individual apolipoprotein resides in the system), fractional catabolic rates (reciprocal of the residence time), and production (synthesis) rates were calculated. The quantitative differences between apoA-I and apoA-II metabolism in male and female subgroups were examined.

[3] Best means to determine the average value and some measure of the variance of this value in the population of interest.

[4] The arithmetic and the geometric mean differ by less than 1.0%. If $X_i = e^{-Lt}$ then L is linear with respect to the geometric mean of X_i's.

TABLE 1.

Characteristic	Female	Male	Total	Units
Number	9	11	20	
Age	21 ± 0.2[a]	22 ± 0.6	21 ± 0.4	yr
Height	168 ± 1.5	180 ± 1.3	175 ± 1.6	cm
Weight	65 ± 1.7	78 ± 3.3	72 ± 2.4	kg
Cholesterol	169 ± 7.1	163 ± 8.9	166 ± 5.8	mg/dl
Triglyceride	93 ± 10.8	92 ± 23.0	92 ± 8.4	mg/dl
VLDL-CH[b]	16 ± 2.2	18 ± 3.9	17 ± 2.3	mg/dl
LDL-CH	103 ± 7.3	105 ± 10.4	104 ± 6.4	mg/dl
HDL-CH	51 ± 4.4	43 ± 3.1	46 ± 2.7	mg/dl
Volume	30 ± 0.9	35 ± 1.3	33 ± 1.0	dl
ApoA-I	124 ± 8.2	108 ± 4.9	115 ± 4.7	mg/dl
ApoA-II	24 ± 0.8	23 ± 1.1	24 ± 0.6	mg/dl

[a] Mean ± standard error.
[b] CH, cholesterol.

RESULTS

Plasma lipoproteins and apolipoproteins A-I and A-II were quantified after 10 days of weight stabilization on an iso-weight diet (**Table 1**). Following the intravenous injection of radioiodinated apolipoprotein A-I or A-II, fasting plasma samples were collected at 10 min, 24 hr, and then daily for 14 days. Non-fasting plasma samples were collected at 6 hr and 12 hr on the first day. Average plasma apolipoprotein radioactivity data normalized to the sample value at 10 min is presented in **Fig. 1** for both apolipoproteins A-I and A-II. Simultaneous studies of apolipoproteins A-I and A-II, conducted in 14 subjects, allowed the calculation of the average difference (apoA-II radioactivity − apoA-I radioactivity) for paired studies (**Fig. 2A**). The probability that the average difference is less than or equal to zero is less than 0.05 for all but the first two data points, indicating the significance of this difference (Fig. 2B).

ApoA-II model

Preliminary data analysis by methods utilizing the area under the curve and correlation coefficients were performed (44). The statistical significant correlation between apoA-II concentration and whole-body residence time and the lack of a significant correlation between apoA-II plasma residence time and apoA-II concentration suggested that there was more than one route

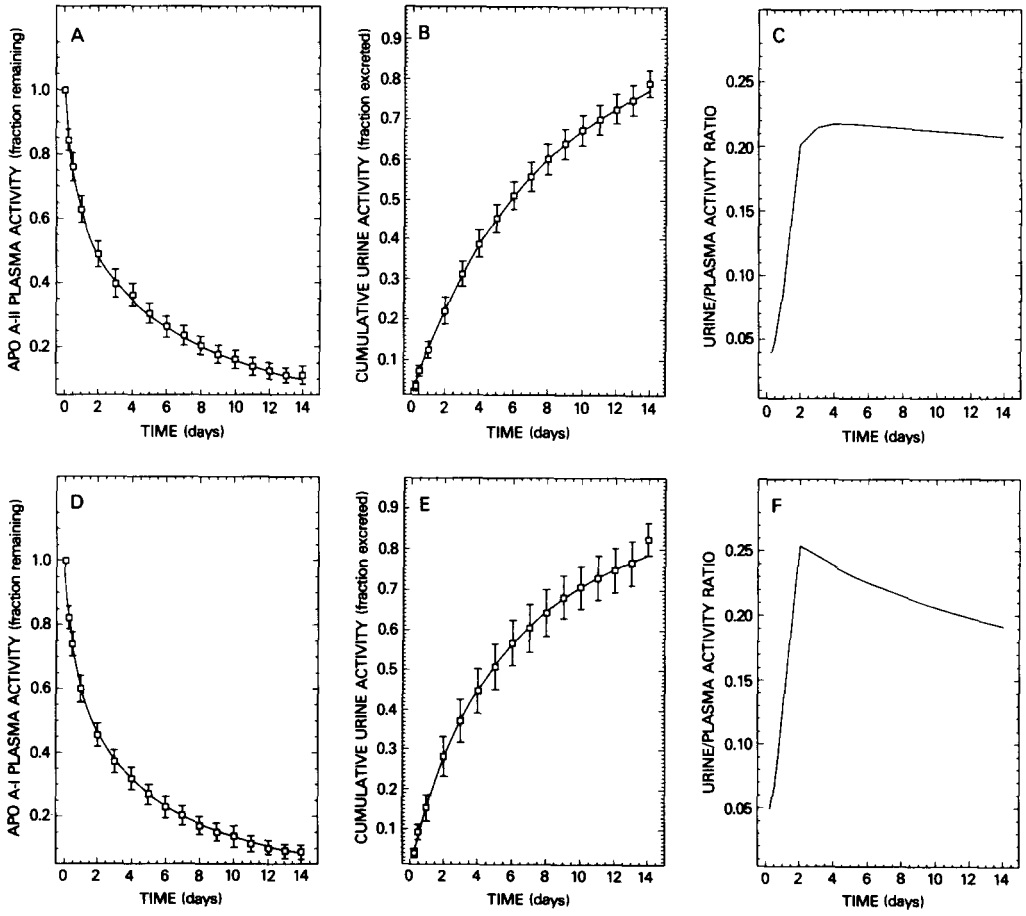

Fig. 1. Plasma, urine, and urine/plasma radioactivity normalized to the 10-min point ± 2 standard errors. A, B, C, apoA-II and D, E, F, apoA-I. Solid lines represent calculated values using the models.

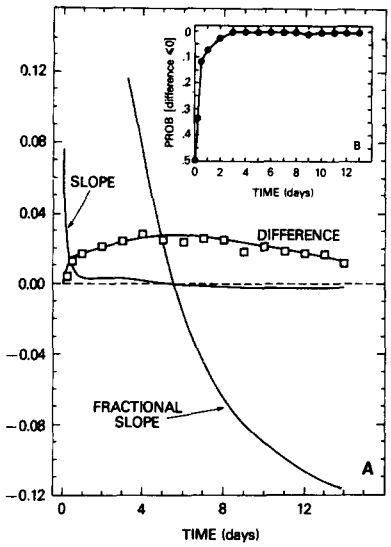

Fig. 2. A, Difference between apoA-II and apoA-I radioactivity. The slope and fractional slope of the difference. B, Probability that difference is less than or equal to zero. Fractional slope of $f(x) = 1/f(x)(Dxf(x))$.

of catabolism for apoA-II. The previous model developed for HDL metabolism by Blum et al. (23) contains two pathways for the metabolism of HDL (**Fig. 3A**). This, in combination with the fact that a large fraction of plasma apoA-II was isolated within HDL and that more than one route of metabolism was suggested by preliminary analysis, indicated that the previous HDL model was a good starting model for analysis of apoA-II kinetics. However, the previous HDL model had the drawback that more than 8% of the radioactivity was unaccounted for in the urine and was lost irreversibly through the path designated L(O, NP) (Fig. 3A). Using this HDL model we were unable to explain the new apoA-II data when plasma, urine, and whole-body observations were considered simultaneously. Specifically, the Blum et al. model predicted decreased counts in the whole-body and urine observations compared to the injected dose. Thus, the model was modified to give a new A-II model (Fig. 3B) which was consistent with the data in Fig. 1. This new apoA-II model has an additional compartment exchanging with the plasma compartment to account for the 8% loss of radiolabeled apoA-II. The resultant urine/plasma ratio is plotted in Fig. 1. The residence time for apoA-II calculated using the model was 5.52 ± 0.05 days and the production rate was 4.3 ± 0.02 mg/dl-day.

The "nonplasma" degradation pathway (Fig. 3B) represents a second metabolic pathway for apoA-II or the lipoprotein that it may be associated with. This second pathway for apoA-II degradation takes one day longer than the most direct pathway and could easily represent degradation by a second separate organ or cell type.

ApoA-I model

Neither the HDL model of Blum et al. (23) nor the new apoA-II model was adequate to explain the mean apoA-I data obtained in the present study. A new apoA-I model was developed by first using the new apoA-II model as a starting apoA-I model. Preliminary analysis

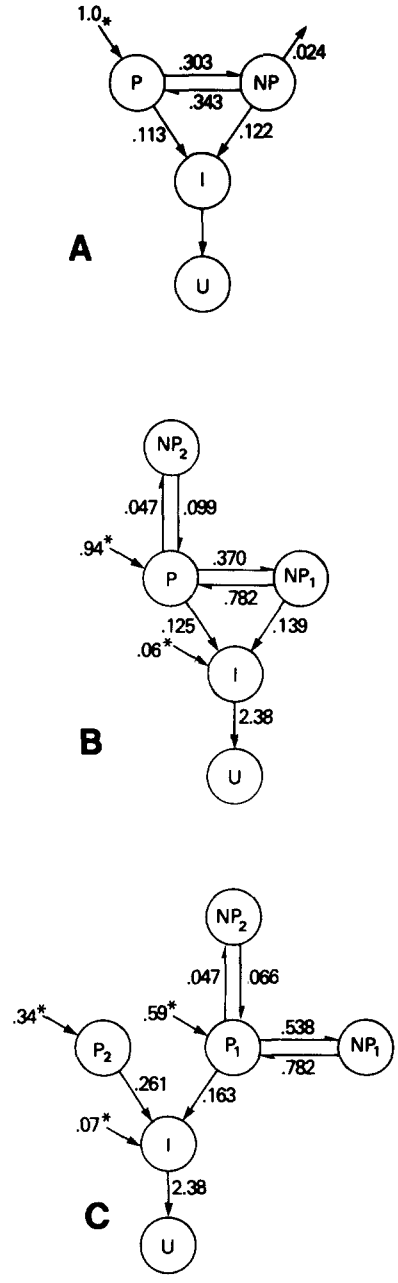

Fig. 3. A, Previous HDL model of Blum et al. (23); B, apoA-II compartmental model; C, apoA-I compartmental model.

(44) indicated a weak relationship between apoA-I concentration and whole-body residence time, but a significant relationship between apoA-I concentration and plasma residence time (44) was observed.

Because of these relationships, the non-plasma decay was decreased in the new apoA-I model. The best fit of the observed apoA-I data resulted when the non-plasma decay was decreased to zero. In addition, it was necessary to include a second plasma compartment that decayed at an increased rate. This need can best be gauged by examining the average difference between apoA-II and apoA-I plotted in Fig. 2A. Clearly, the apoA-I decays at a faster rate than apoA-II for the first few days. The simultaneous fit of the apoA-I plasma, urine, and whole-body observations and the difference in plasma curves are best supported by the simple apoA-I model illustrated in Fig. 3C. The urine/plasma radioactivity ratio was calculated using the apoA-I model and is plotted in Fig. 1. The residence time for apoA-I calculated using the model is 5.04 ± 0.08 days and the production rate is 22.8 ± 0.4 mg/dl-day.

Further examination of the A-I model focused on the ratio R of the decay from compartment P_2 and compartment P_1. To establish the sensitivity (52) of the fit to the data as well as the remaining parameter values to R, the ratio was constrained. The ratio R = L(I, P_2)/L(I, P_1) was fixed at several values in the neighborhood of R = 1.6[5] and the error relative to R = 1.6 was determined for the simultaneous fit of the data (plasma, urine, and whole-body) by adjusting all parameters except L(I, P_2). This determines the best value of R as well as providing a measurement of sensitivity for several parameters to the ratio R. The results of this examination are plotted in **Fig. 4.** The relative error (error divided by the error at the nadir) takes a substantial dip at 1.6, therefore this value of R was chosen as the best value. Thus, R = 1.6 is the best value in that it gives a better resolution of error. The error is much larger when the residence times for the two fractions are equal indicating the need for a second apoA-I component in this new model. In addition to the residence time, the fraction F of the initial radioactivity that must go into compartment P_2 was determined with the remaining radioactivity going into compartment P_1. The sensitivity of F with respect to changes in the total relative error is also obtained and plotted in Fig. 4. The isolation of lipoprotein fractions containing different amounts of either the fast or slow component of apoA-I may be expected to have a different R. This analysis indicates

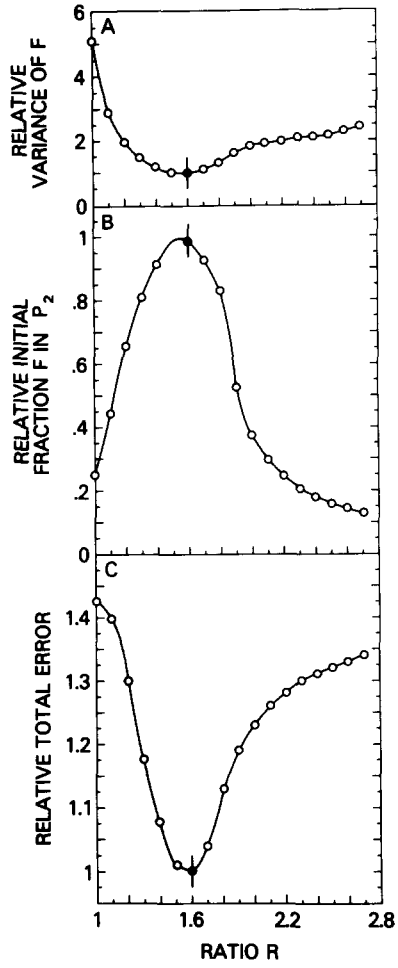

Fig. 4. Relative sensitivity of apoA-I model to parameter ratio R = L(I, P_2)/L(I, P_1). A, relative error (predicted-observed) as a function of R. Smallest at R = 1.6; B, relative fraction F of injected radioactivity in compartment P_2; C, relative coefficient of variation of F as a function of R.

that the kinetics of apoA-I are very sensitive to changes in R and that if different fractions do have a different R their kinetics may also differ.

Male and female subgroups

The data were divided into male and female subgroups and the average data is presented in **Fig. 5.** Using the new models, separate apoA-I and apoA-II models were developed for both subgroups. The topology (connectivity) for both the apoA-I and apoA-II models remained unchanged. Changes in several rate constants were necessary to provide adequate interpretation of the observed data in each subgroup. The resulting kinetic parameters are shown, and the observed and calculated values are plotted in Fig. 5. Differences between apoA-I and apoA-II metabolism were observed in both plasma and urine from each sex. The second

[5] This is an iterative (bootstrap) procedure in which, on the first iteration, the best value is determined. From that point on, all determinations are made in a neighborhood of the best values.

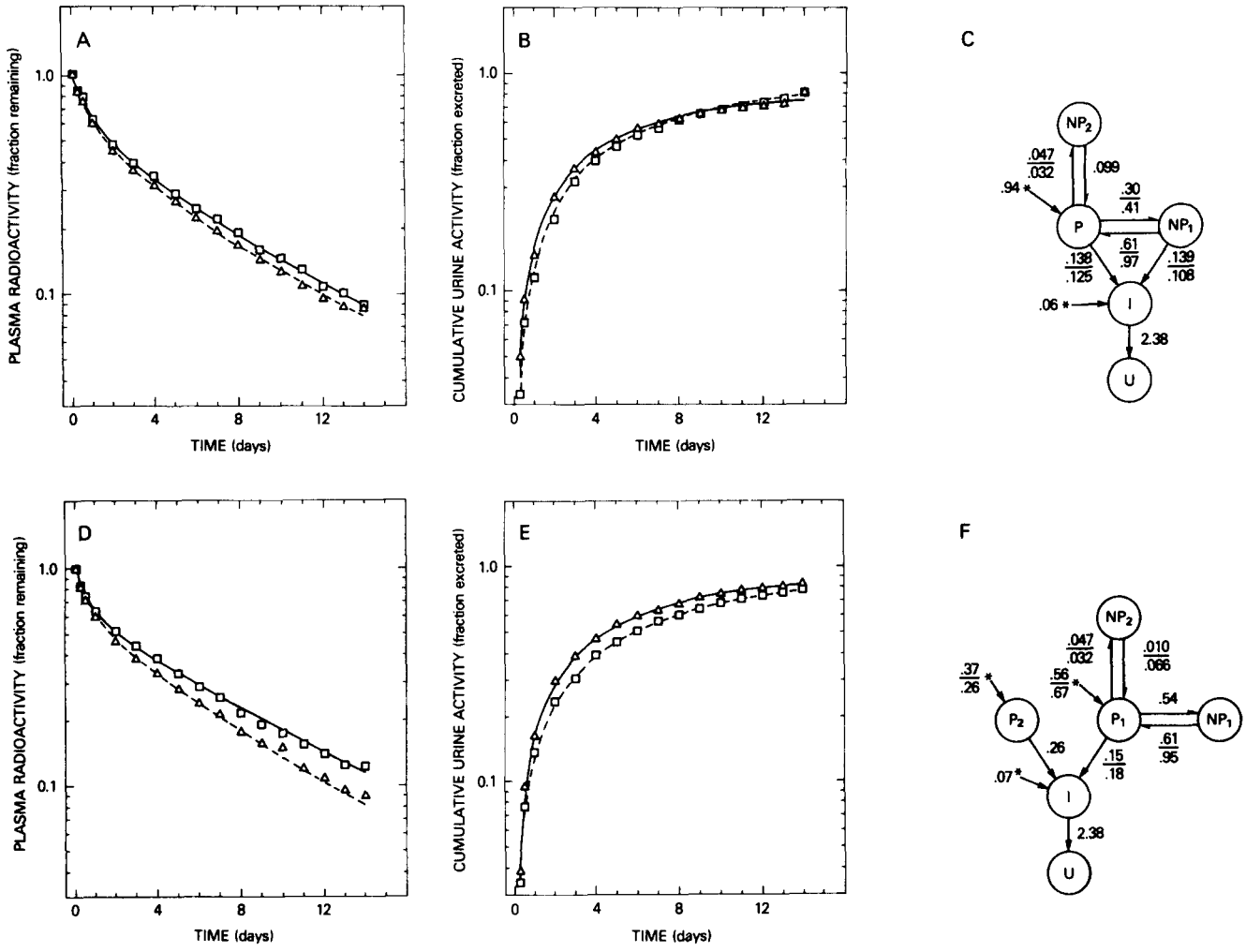

Fig. 5. Plasma and urine radioactivity normalized to the 10-min point, and compartmental models with female/male kinetic parameters. A, comparison of plasma apoA-I (△) and apoA-II (□) in females; B, comparison of urine apoA-I (△) and apoA-II (□) in females; C, apoA-II model; D, comparison of plasma apoA-I (△) and apoA-II (□) radioactivity in males; E, comparison of apoA-I (△) and apoA-II (□) urine radioactivity in males; F, apoA-I compartmental model.

decay route of apoA-II through the non-plasma pathway accounted for 25% of the apoA-II decay in males; material in this pathway took 0.9 days longer to reach the urine compared to primary or major pathway for degradation of ApoA-II. In the female subgroup, 29% of the apoA-II decayed by this route and took 1.3 days longer than the apoA-II decaying through the most direct route. Even though the residence time of the second component (P_2) of plasma apoA-I is equal for males and females, over 40% of the apoA-I is found in this component for females while only 28% is found in males. Production rates for apoA-I were 24 mg/dl-day in female subjects, somewhat larger than the 22 mg/dl-day calculated in males. However, a production rate of 4.7 mg/dl-day of apoA-II in females was 20% larger than the corresponding value in males of 3.8 mg/dl-day.

These studies were interpreted as indicating that apoA-I and apoA-II were metabolized separately. In addition, differences exist in apoA-I and apoA-II metabolism of males and females emphasizing the physiological significance of these pathways.

ApoA-I and apoA-II concentrations

The average steady-state values for apoA-I and apoA-II concentrations are tabulated in Table 1. The concentration data were further examined to answer two questions: *1*) Are the subjects in steady state during the turnover portion of the study? and *2*) was there any evidence that the apolipoprotein concentrations were influenced by food consumption that occurred prior to the 6- and 12-hr plasma samples?

In all subjects apolipoprotein concentrations were plotted as a function of time. The best (minimal least square error) straight line was determined for each subject and each apolipoprotein. The standard residuals (error/standard deviation of error) were plotted and tested for normal distribution using the Kolmogorov-

Smirnov test (50, 51) as a measure of the correctness of the statistical model. In addition, the slope (B_1) of the regression line through the data was calculated and the probability that the absolute value of (B_1) was less than zero was determined. The cumulative frequency distributions of these probabilities are plotted in **Fig. 6** for the 14 subjects in which both apoA-I and apoA-II were studied simultaneously. At any level of significance apoA-II has a larger number of studies in steady state.

The frequency distribution of the standard residuals for the fasting and nonfasting plasma apoA-I and apoA-II concentrations are plotted in **Fig. 7A.**

There was little difference between the distribution of fasting and nonfasting standard residuals for apoA-II indicating that apoA-II is not only constant over the 14-day period of the study but is stable over the short-term period when the subjects were nonfasting. In contrast, the distributions of nonfasting apoA-I standard residuals were shifted 0.5 standard deviations to the left of the fasting residuals (Fig. 7B). The distribution of the nonfasting residuals has the same shape as the distribution of the fasting residuals. This indicates that on the average apoA-I concentration was elevated when the nonfasting values were compared to fasting values.

Thus, in addition to differences in the metabolism of apoA-I and apoA-II, there are differences in the concentration of apoA-I but not apoA-II in response to dietary pertubations. In the Discussion, we compare and contrast the kinetics of apoA-I and apoA-II and consider several physiologic mechanisms that may lead to these findings.

DISCUSSION

In this study three important differences between apoA-I and apoA-II metabolism were observed and quantified. These differences will be discussed in order of their occurrence in the turnover of apolipoprotein A-I and A-II.

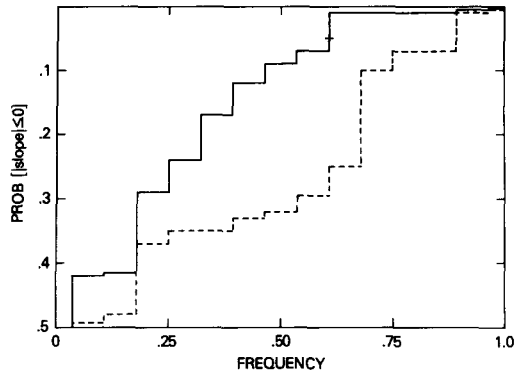

Fig. 6. Cumulative frequency distributions in which the probability of the absolute value of the slope of a line through the steady-state values of apoA-I and apoA-II is less than zero. A probability of 0.5 indicates no detectible slope. Sixty percent of the subjects have better than a 5% chance of having a slope less than zero for apoA-I (——), while 85% of apoA-II (---) subjects have a 5% chance of a slope less than zero.

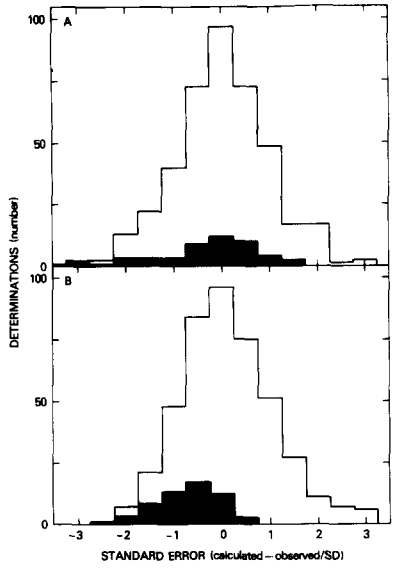

Fig. 7. Frequency distribution of standard error for fasting and nonfasting apoprotein concentrations. A, For apoA-II, both fasting and nonfasting values have the same distribution. B, For apoA-I, the nonfasting mean standard error is shifted to the left indicating that nonfasting apoA-I values are elevated on the average.

First ten minutes

The examination of the metabolism of apolipoproteins is the study of interactions of these apolipoproteins in vivo. Interesting and significant metabolism may occur in periods of a few seconds or a few weeks. The time scale in metabolic studies is determined by the experimental design through a set of rules known collectively as "sampling theory" (54). Based on previous HDL studies, the unit of time in which interesting and significant apoA-I and apoA-II metabolism occurs is days and this study was designed accordingly. Frequent samples were taken in the first day so that kinetic parameters could be resolved (i.e., delays) up to 0.24 days, and thereafter the sampling frequency was decreased to daily with a subsequent decrease in resolving power of the experimental method to the desired level. Because of this careful experimental design, no information is lost regarding the topology of the model when each set of data is normalized to unity at the 10-min sample point. In addition, this puts the data in a form which is easily comparable to data in the literature. By developing the model and then extrapolating back to zero, several interesting points can be examined. First, in the initial 10-min period following injection, 6% of the labeled apoA-II, and 7% of the labeled apoA-I moved from the space of distribution for the apolipoprotein to the space of distribution containing the iodide pool. There is, however, no information regarding the

rate at which this change of space occurred, except that by 10 min it had taken place.

If the initial movement of label into the iodide space took place quickly (almost zero time with respect to 10 min) then this radioactivity may be associated with a component that distributes in the same space as the iodide pool. However, if the movement took place slowly (throughout the 10-min period), then it may represent an interaction of this component with the system. In the first case it probably represents an artifact of the study, but in the second case this 6–7% may represent true metabolism, the details of which were not resolved by this experimental design.

Ten minutes to three days

A major difference (Fig. 2) in the kinetics between apoA-I and apoA-II exists in the 10-min to 3-day period. This observation persists after the differences that occur in the first 10 min of the experiment are eliminated by normalization. A large portion of this difference is represented by the component P_2 in the apoA-I model depicted in Fig. 3C. The rate of change and the fractional rate of change of this difference between apoA-I and apoA-II metabolism are also plotted (Fig. 2). The estimated probability that the discrepancy between apoA-I and apoA-II could be less than or equal to zero from Fig. 2B is small, indicating that the difference is not due to random error. The positive slope of the difference curve was resolved since the second plasma component, P_2 in the apoA-I model, has a residence time that is 1.6 times the residence time of apoA-I in compartment P_1 and 1.4 times the residence time of apoA-II.

The last ten days

A third difference between apoA-I and apoA-II kinetics can be best detected by examination of the difference between the terminal slope of apoA-I and apoA-II radioactivity. Because this is equal to the slope of the difference,[6] one can determine from Fig. 2 that the apoA-II slope is greater at later time points following the peak of the curve. The results indicate that the remaining radioactive apoA-II is decaying faster than the remaining apoA-I radioactivity.

Sex differences

The above observations exist for apoA-I and apoA-II kinetics in the group taken as a whole as well as for subgroups divided by sex. The observation that 40% of the apoA-I decays from the fast component in females compared to 28% in males, even though this component has the same residence time for both, is of interest. This observation, combined with the evidence that males have increased VLDL-cholesterol and decreased HDL-cholesterol fractions (55), indicates the fast pool for apoA-I may be linked to HDL_{2b} metabolism.

The production (synthesis) rate in mg/dl-days of apoA-I is 7% greater in females. In other words, if males and females were matched with respect to volume, then the apoA-I production rate in females would be somewhat above males. These results may be interpreted as indicating that females produce less apoA-I protein per day, however, because the pool size is smaller in females, the plasma concentration is elevated. The same interpretation holds if production (synthesis) rate is normalized by weight. The importance of the plasma concentration on transport rate could ultimately best be examined using the clamp technique. These types of studies are not feasible in humans. $L(P_1, NP_2)$ need only be changed in the female subgroup indicating a large change in the residence time and mass ratio of NP_2. Because of the magnitude, this change is interpreted to indicate that compartment NP_2 represents a complex system of compartments, the details of which cannot be realized[7] with the present data.

ApoA-I and apoA-II concentrations

The nonfasting mass of apoA-II has the same mean distribution as the fasting values. In the same subjects the distribution of nonfasting plasma apoA-I mass is elevated one-half standard deviation above the distribution of fasting values. These results indicate that specific activity determined at nonfasting points are underestimated because of increased apoA-I mass. This is a result which would not have been detected if specific activity data had been examined. These results are not unexpected in view of the previous studies demonstrating that the intestine is a major source of apoA-I biosynthesis (36–39). The lack of change in apoA-II suggests that a significant quantity of apoA-II may not be made in the intestine or that apoA-II synthesis in the intestine is not subject to dietary modulation. We do not interpret this to indicate that apoA-II is never made in the intestine or become subject to dietary modulation in pathologic states such as in reference 56.

In addition, the steady-state assumption of constant apolipoprotein mass was examined and apoA-I was less constant than apoA-II over the 14-day study period. Since weight and diet were controlled and because no similar changes were observed with apoA-II, apoA-I may be modulated by additional factors which do not affect apoA-II.

The combined results presented in this report indicate that the metabolism of apoA-I and apoA-II are different and complex. In the present study, apoA-I and apoA-II were modeled and the similarity of the two

[6] $Dx(f(x) + g(x)) = Dx(f(x)) + Dx(g(x))$ (69).

[7] Realization is the process of determining the structure of a system from the examination of the system responses to a known input.

models was examined. The previous HDL model of Blum et al. (23) contains portions of both the apoA-I and apoA-II models. The HDL model, however, did not contain a second compartment for apoA-I as was required in the present model. The finding that apoA-II is catabolized faster than the slow component of apoA-I is also different than previously reported, and is represented by the additional degradation pathway in the apoA-II model. Blum et al. (23) also required a similar pathway; however, the present study indicates that it exists for apoA-II only.

The recognition of the complexity of the metabolism of apoA-I and apoA-II is not unexpected. The physiochemical polydispersity of plasma lipoproteins has been well established by ultracentrifugation and electrophoretic techniques. HDL has been separated into three major components, HDL_{2b}, HDL_{2a}, and HDL_3 by ultracentrifugation (57) and into four components by gradient gel electrophoresis (58). As noted above, HDL_2 appears to be influenced to a greater extent than HDL_3 by hormones (59) and the metabolism of triglyceride-rich lipoproteins (60). Differential rate of catabolism of HDL subfractions was also noted in Tangier patients following the injection of HDL (d 1.063–1.21 g/ml). In these patients HDL_{2b} was catabolized at a faster rate than HDL_{2a} and HDL_3, suggesting a difference in metabolism of HDL subfractions.

In addition to apolipoproteins A-I and A-II, both E and the C apolipoproteins have been isolated from various HDL fractions. ApoE has recently been shown to have a plasma residence time of less than 0.5 days (61, 62). Several of the C apolipoproteins have been reported to have a residence time of less than 2 days (63). In contrast, the residence time of apoA-I and apoA-II are much longer. Since all of these apolipoproteins can be isolated from the HDL fraction, it is reasonable to expect several separate fractions of HDL with different residence times. Within this context, it is important to determine which parts of the apoA-I and apoA-II model are enough alike, topologically, to propose that they are on a subfraction. The fast component of apoA-I and the second decay path of apoA-II are the portions of the kinetics so different as to suggest that each may be on a class of separate lipoproteins. ApoA-I has been isolated in the lipoprotein fraction with density less than 1.063 g/ml in several studies (64, 65). In addition, when normal radiolabeled HDL was injected into subjects with Tangier disease, a major portion of apoA-I radioactivity and mass was isolated with the lipoproteins of density less than 1.063 g/ml (66). These results suggest that a portion of the second plasma component P_2 in the model for plasma apoA-I may represent the kinetics of apoA-I associated with triglyceride-rich lipoproteins (e.g., plasma chylomicrons and VLDL).

It has been speculated that radioiodinated proteins may show minor differences in physicochemical properties depending on which and how many amino acid residues are iodinated (67). We further postulate that this variability in labeling may account for the second species of radiolabeled apoA-I which has a separate catabolic fate. Such an isotopic effect must, however, be a minor contributor to P_2; otherwise, it becomes difficult to understand why the fast apoA-I component does not need to exchange with an extravascular component to explain the observed data. This observation can easily be explained if the fast component of apoA-I is associated with a triglyceride-rich lipoprotein such as VLDL or HDL_2 which may not have a large extravascular circulation.

Recently, others have reported that a difference exists between apoA-I and apoA-II when labeled endogenously (68), indicating that the above observations are probably not artifacts of labeling. Undoubtedly, all the explanations account for a portion of the observations; however, the distribution of apoA-I with triglyceride-rich lipoproteins certainly represents an important part of the explanation, as does the existence of an apoA-I-rich fraction of HDL with rapid turnover.

In the present study, we have utilized a number of experimental designs (injection of radiolabeled apolipoprotein, large number of normal subjects, simultaneous study of apoA-I and apoA-II, collection of plasma data without separation, collection of urine and whole-body data) and analytical techniques (statistical techniques, comparison of simultaneous studies, compartmental models, simultaneous consideration of complementary data) that have enabled us to examine the kinetic heterogeneity of both apoA-I metabolism and apoA-II metabolism and propose the following. *1*) There are major differences between the kinetics of apoA-I and apoA-II. *2*) There are two plasma components participating in apoA-I metabolism that can be detected because they decay at different rates; there is only one plasma component for apoA-II. *3*) There are two distinguishable routes of metabolism participating in apoA-II kinetics that can be detected because metabolism takes longer in one path than the other. *4*) Only one pathway of catabolism is common to both apoA-I and apoA-II. *5*) This heterogeneity between apoA-I and apoA-II is detected in both male and female subgroups. *6*) Fasting apoA-I concentrations are more variable than fasting apoA-II concentrations. *7*) ApoA-II concentrations are less responsive to dietary change than are apoA-I concentrations.

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